application

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Liquid Chromatography/Mass Spectrometry | Natural Products

Analysis of Anatoxin-a in Drinking Water by Automated On-line Derivatization Electrospray LC/MS

Introduction

Anatoxin-a (2-acetyl-9-azabicyclo[4,2,1]non-2-ene) is an alkaloid neurotoxin produced by a number of blue-green algae (cyanobacteria) including *Anabena flos-aquae, Aphanizomenon flos-aquae and Oscillatoria.*[1] The current interest in anatoxin-a derives from an increasing number of reports of animal deaths following algal bloom ingestion and from concerns about drinking water quality.

A number of chromatographic methods, including HPLC/UV and GC/MS, are available for the analysis of anatoxin-a in water blooms.[2] In HPLC analysis, an ion-pair reagent is typically used with a reversed-phase column. However, the use of an ion-pair reagent reduces sensitivity. In this study, an automated on-line derivitization LC/MS method was developed to determine trace levels of anatoxin-a in drinking water. Based on 9-Fluorenyl its rapid reactivity with anatoxin-a methylchloroformate (FMOC) was selected as the derivatizing agent. See Figure 1.

Experimental

Anatoxin-a hydrochloride was purchased from Sigma Aldrich Japan (Tokyo, Japan). A stock solution was prepared by dissolving the anatoxin-a in HPLC grade methanol. Samples were prepared by spiking drinking water. The SDB-RPS disk (47 mm, Empore, 3M, USA) was used to provide rapid sample preparation.

FMOC (HP Part No. 5061-3337) was used for derivitization. A 0.4 N, pH 10.4 borate buffer (HP Part No. 5061-3339) was used for pH control. Deionized water was used as the needle wash.

Derivatization with FMOC was done by running the following program on the Agilent 1100 Series autosampler.

1. Draw 20 µl from the borate buffer vial
2. Draw 0 µl from the water vial to rinse the outside of the needle
3. Draw 1 µl from the FMOC vial
4. Draw 0 µl from the water vial
5. Draw 10 µl from sample vial
6. Mix with 30 µl of air; cycle 5 times
7. Inject 10 µl

Two-hundred-ml aliquots of blank drinking water were spiked at 1, 2, 4, 10, 20, and 40 ng of anatoxin-a. Each 200-ml aliquot was adjusted

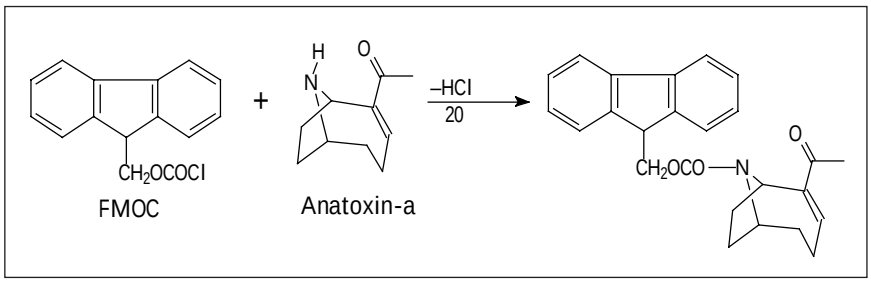

Figure 1. Chemical reaction of anatoxin-a with FMOC.

to pH 10 with sodium hydroxide. The sample was applied to the SDB-RPS disk, which had been preconditioned by washing with 20 ml of methanol and 20 ml of water. The disk was eluted with 10 ml methanol. This extract was concentrated to 1 ml under nitrogen at 50 psig. A 10-µl portion of the extract was injected into the LC/MSD system. Ammonium acetate was used in the mobile phase to provide good peak shape.

The system consisted of an 1100 Series binary pump, thermostatted column compartment, vacuum degasser, autosampler, and LC/MSD. The LC/MSD was used with the electrospray ionization (ESI) source. Complete system control and data handling were provided by the ChemStation for LC/MS.

Results and Discussion

The anatoxin-a standard was first analyzed in scan mode to determine the retention time and mass spectrum. Figure 2 shows the total ion chromatogram (TIC) and mass spectrum at 1 µg/ml. The derivatized anatoxin-a could be detected without interference from the derivatization reagent (FMOC). The mass spectrum of the derivatized anatoxin-a shows the protonated ion $[M+H]^+$ at m/z 388 and a sodium adduct ion $(M+Na)^+$ at m/z 410.

Analysis of anatoxin-a in drinking water was performed in SIM mode to maximize sensitivity and to reduce sample matrix interferences. The base ion (m/z 388) for the derivatized anatoxin-a was selected for monitoring.

Figure 3 shows that the SIM chromatogram of anatoxin-a spiked into blank drinking water at 10 pg/ml indicates detection at a sub-ppt level without sample matrix interferences. The method shows good recovery and RSDs for the range of 5–200 pg/ml. See Table 1.

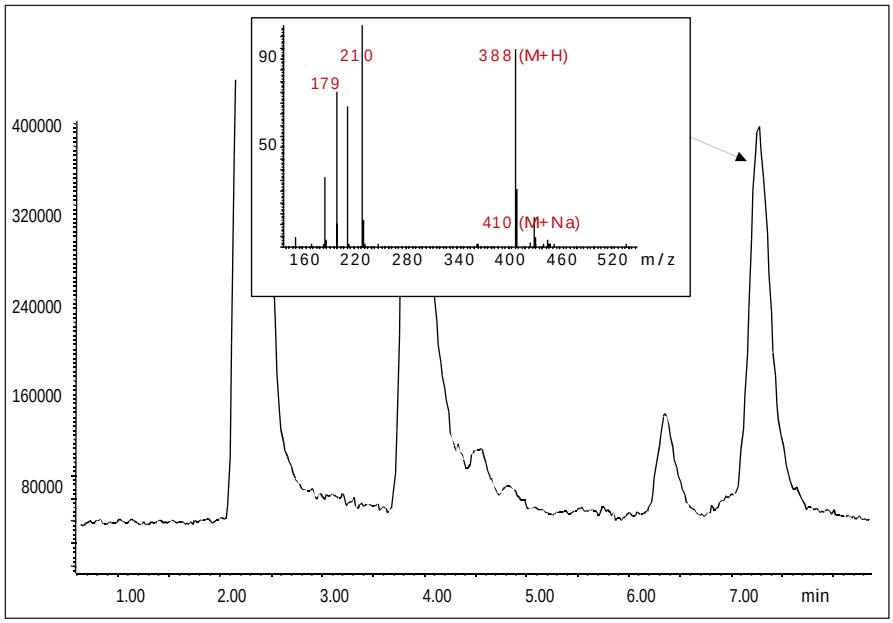

Figure 2. Total ion chromatogram and mass spectrum of derivatized anatoxin-a (1 µg/ml).

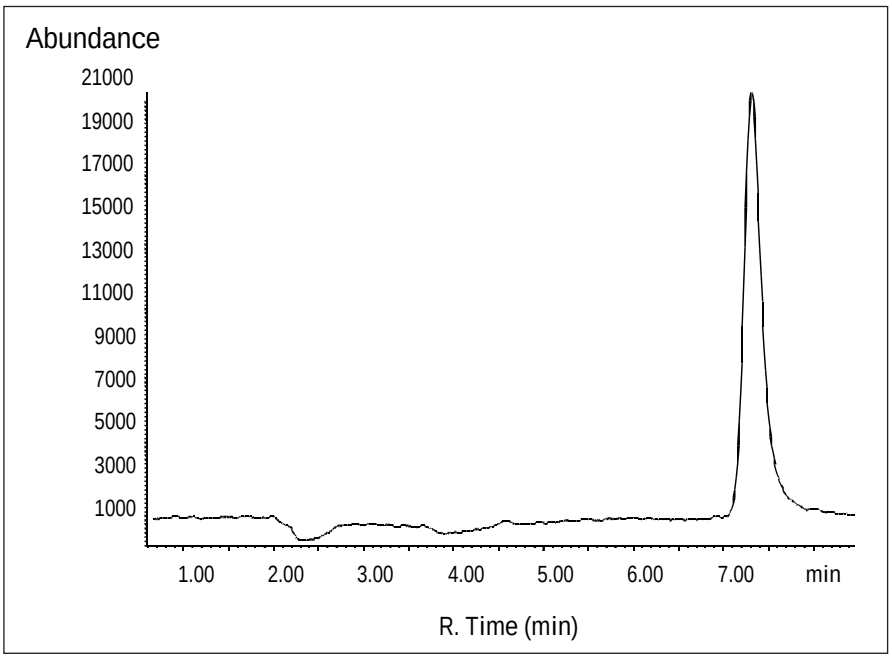

Figure 3. SIM chromatogram of derivatized anatoxin-a in drinking water (10 pg/ml).

Table 1. Recovery of anatoxin-a.

Spiking Level pg/ml	Recovery %	RSD (n = 5) %
200	88.1	5.3
100	84.5	5.4
50	85.3	5.8
20	75.7	7.2
10	77.9	12.5
5	78.1	17.9

Chromatographic Conditions	
Column:	Inertsil ODS3 (5 um, 150 mm, 2.1 mm)
Column Temp:	40°C
Mobile phase:	A = 50 mM ammonium acetate B= Acetnitle 50% A/B
Flow rate:	0.2 ml/min
Injection vol:	10 µl
MS Conditions	
Source:	ESI
Ion mode:	Positive
Vcap:	4000 V
Nebulizer:	50 psig
Drying gas flow:	10 l/min
Drying gas temp:	350°C
Scan range:	100–600 amu
Step size:	0.1
Peak width:	0.15 min
Time filter:	On
Fragmentor:	100 V

Figure 4 shows the calibration curve for 5–200 pg/ml of anatoxin-a with a good correlation coefficient ($r^2 = 0.998$).

Conclusion

The automated on-line derivatization-LC/MS method allows highly selective and sensitive detection of anatoxin-a in drinking water. This method provides a low detection limit which is less matrix-dependent than standard methods without derivitization. The method was successfully applied to the analysis of anatoxin-a in drinking water; it provided a detection limit below 10 pg/ml.

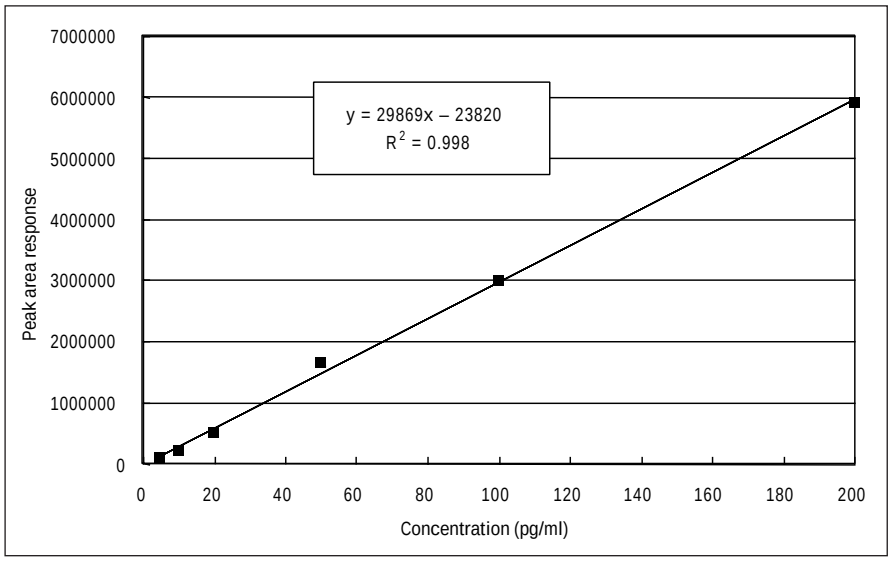

Figure 4. Calibration curve for anatoxin-a in drinking water.

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Flow rate:	0.2 ml/min
Injection vol:	10 µl
MS Conditions	
Source:	ESI
Ion mode:	positive
Vcap:	4000 V
Nebulizer:	50 psig
Drying gas flow:	10 l/min
Drying gas temp:	350°C
SIM ions:	m/z 388
Peak width:	0.15 min
Time filter:	On
Fragmentor:	100 V

References

1. Mahmood, N. A.; and Carmichael, W. W.: Paralytic Shellfish Poisons Produced by the Freshwater Cyanobacterium *Aphanizomenon flos-aquae* NH-5. Toxicon.,vol. 24, 1986, pp. 175–186.
2. Park, Ho-Dong; Watanabe, Mariyo F.; and Harrada, Kenichi: Hepatotoxin (Microcystin) and Neurotoxin (Anatoxin-a) Contained in Natural Blooms and Strains of Cyanobacteria from Japanese Freshwaters. Natural Toxins, vol. 1, 1993, pp. 353–360

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